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Influence of Repeated Washings with Soap and Synthetic Detergents on pH and Resident Flora of the Skin of Forehead and Forearm

Results of a Cross-over Trial in Healthy Probitioners

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Ten healthy individuals washed their forehead and forearm twice a day over consecutive periods of four weeks with soap and synthetic detergents or vice versa (cross-over design). In general the pH values were higher during the period when soap was applied (the mean pH differed by 0.3 units, $p<0.01$). As a rule the counts of coagulase-negative staphylococci were not much altered. The number of propionibacteria, however, was markedly higher when soap was used ($p = 0.02$ and 0.01 resp.). At the forehead there was a clear correlation between bacterial counts and skin pH both with propionibacteria (0.56, $p<0.001$) and staphylococci (0.51, $p<0.001$). At the forearm only the former proved true (0.24, $p<0.05$). Thus the skin pH seems to be open to long-standing changes according to the preferred washing habits which may also be of major influence on the composition of the cutaneous bacterial flora. *Key words: Skin pH; Bacterial flora of the skin; Soap; Synthetic detergents.* (Received March 3, 1986.)

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The effect of soaps and synthetic detergents on the pH and bacterial flora of the skin has repeatedly been investigated. Most of the pertinent investigations, however, concentrated on the effect of single washings with soap alone (1, 2) or soap and synthetic detergents (3, 4) solely on the skin pH. Only rarely interest was focused on corresponding data for repeated washings (1, 4). Investigations of the effect of synthetic detergents (5) and soap and synthetic detergents (6) on the bacterial flora of the skin have not frequently been undertaken either.

The importance of the acidity of the skin for its bacterial flora has already been an essential part of the "acid mantle" concept created by Marchionini (7). Later Arnold (2) has supported this theory by his experiments on the influence of artificial acidification and alcalinization of human skin on its bacterial flora, and it is still generally accepted (8).

Parallel investigations of the effect of repeated washing procedures on both skin pH and resident flora at the same time, however, have seemingly not been undertaken so far, especially not in a defined cross-over design allowing intraindividual comparisons. The results of such a study will be presented in the following.

MATERIAL AND METHODS

Study population

Five male and five female healthy probitioners entered the study after written informed consent. Their age ranged from 21 to 38 years, the mean age amounting to 28 years. Five of them were attributed to a

group which started defined washing procedures with soap (Lux-soap, Lever-Sunlicht, Hamburg, FRG), the other five started using synthetic detergents (Sebamed flüssig, Sebapharma, Boppard, FRG) (later referred to as "Lux group" and "Sebamed group").

pH determination

The skin pH was determined by means of the flat glass electrode for surface measurements developed by Ingold and clinically evaluated by Schirren (9) connected to a precision pH-mV-meter (pH 521, WTW, Weilheim, FRG). The measurements themselves were performed due to standard methods (10). Each measurement was performed three times and the mean value taken.

Investigation of the bacterial flora

The bacterial flora was sampled by the detergent scrub method developed by Williamson & Kligman (11) and proven most efficient for the analysis of human skin flora by Hartmann (12). Defined specimens were repeatedly diluted and inoculated on Columbia agar (BBL, Heidelberg, FRG) with 5% defibrinated sheep blood and tryptic soy agar (BBL, Heidelberg, FRG) and incubated for two and seven days at 37° centigrade in aerobic and anaerobic (GasPak jars with hydrogen and carbon dioxide generator kits, BBL, Heidelberg, FRG) atmosphere respectively. Among the bacteria grown under aerobic conditions coagulase-negative staphylococci were identified by gram stain, negative plasma coagulase test and biochemical reactions (API STAPH, bioMerieux, Nürtingen, FRG) (14, 15) and also quantitated.

Washing procedures

Washing of the skin with soap or synthetic detergents was performed twice a day (in the morning and in the evening) at the forehead and the proximal part of the flexor site of the forearm for two minutes by the probitioners themselves.

Time course of investigations

At the beginning of the trial both skin pH and bacterial flora were repeatedly investigated (day 1 to 3 and 1 and 3 respectively). After the skin areas under consideration were either cleaned with soap or with synthetic detergents over a period of four weeks (day 4 to 31). From day 32 to 59 these procedures were continued in the same way, but with the type of preparation not used during the first half of the trial (cross-over design allowing both interindividual and intraindividual comparisons). During the whole washing period both skin pH and bacterial flora were determined at the forehead and the forearm every seventh day. The investigations themselves were performed in the middle of the application interval, i.e. several hours after the last washing procedure. In addition, the skin pH values were once recorded just before and after a washing procedure and every half hour thereafter during a period of four hours (this was done in the last week of the second half of the trial).

Mathematical and statistical analysis

The pH being the negative decadic logarithm of the hydrogen ion concentration it is not possible to calculate the arithmetic mean of pertinent data directly. Instead the figures found have to be delogarithmicated again after. For the same reason no standard deviation data can be visualized in a graph based on a pH scale. For statistic evaluation the following procedures were applied: Wilcoxon's test for tied pairs, Wilcoxon's test for the comparison of two independent samples, determination of the correlation coefficient of Bravais & Pearson.

RESULTS

pH values

Before the beginning of the washing procedures the pH values at the forehead and at the forearm lay in the range from 4.48 to 5.63 and 4.22 to 5.44 respectively. The greatest difference between two recordings in any of the individuals amounted to 0.94 (forehead) and 0.53 (forearm). The mean values amounted to 4.80 and 4.68. The differences between the probitioners of each of the two groups were negligible. The mean values in the "Lux" and "Sebamed" group amounted to 4.80 and 4.80 with reference to the forehead and 4.61 and 4.77 with reference to the forearm.

In all individuals, starting the trial with soap, the skin pH increased markedly both at the forehead and at the forearm. After the change to synthetic detergents the pH values

decreased again. In the individuals using synthetic detergents first the pH values remained more or less stable during the first half of the trial, in between the data showed a tendency rather to decrease. After the switch to soap the pH increased reaching values beyond the initial level (Figs. 1 and 2).

Comparing all data acquired during the application of soap with those related to the application of synthetic detergents the skin pH proved to be higher during the application of soap by 0.3 pH units ($p<0.01$). The difference in the skin pH between probitioners using soap and those using synthetic detergents became significant at the end of the second week of application ($p<0.01$) and remained so for the rest of the application period ($p = 0.02$).

In the short run under steady-state conditions the application of both soap and synthetic detergents led to an initial increase of the pH which, however, was much more marked after soap application ($p = 0.01$). Both after the use of soap and synthetic detergents the pH values moved back to about the initial level during approximately two hours' time (Figs. 3 and 4).

Bacterial flora

In general the number of coagulase-negative staphylococci per square centimetre showed no clear tendency to change under repeated washings with soap or synthetic detergents. Solely at the forehead these germs clearly increased in the period of soap washings as compared to a previous period of synthetic detergents washings ($p = 0.05$; Figs. 5 and 6). It may, however, be true, that systematic washing irrespective of the cleasing agent leads to an increased colonisation of the skin with coagulase-negatice staphylococci as it seems to be implied by these figures.

With the propionibacteria which almost entirely belonged to the species Propionibacterium acnes and Propionibacterium granulosum the situation turned out to be totally different. Under repeated washings with soap the propionibactera counts rose dramatically (by about one order of magnitude). After a change to synthetic detergents they fell again (with respects to the forehead in an especially rapid manner) to reach final levels even below the initial ones. Under repeated washings with synthetic detergents the number of propionibacteria was markedly reduced, but after a switch to soap this tendency was totally altered, and at the forearm even bacterial counts much higher than the initial ones were found (Figs. 7 and 8). To sum up: skin repeatedly washed with soap harbours much more propionibactera (forehead: $p = 0.02$, forearm: $p = 0.01$). At the forehead the difference becomes significant at the end of the second week ($p<0.1$) and remains so during the next two weeks ($p = 0.02$, $p = 0.02$). At the forearm the difference already becomes significant at the end of the first week ($p<0.01$) to remain so thereafter ($p<0.05$, $p<0.01$, $p<0.01$).

Interdependence of bacterial counts and pH

At the forehead both the number of coagulase-negative staphylococci and the number of propionibacteria were clearly correlated with the skin pH. The correlation coefficients amounted to 0.51 ($p<0.001$) and 0.56 ($p<0.001$). At the forearm a corresponding correlation could be found only with the propionibacteria (0.24, $p<0.05$) but not with the staphylococci (the correlation coefficient being 0.02).

DISCUSSION

The pH values found in the present investigation at the forehead and forearm are well within the range of other pertinent data published in the past (10). Some of the mean values for the skin pH published in earlier papers, however, make the skin surface look more alkaline than it is, due to improver mathematical handling of individual data (compare the

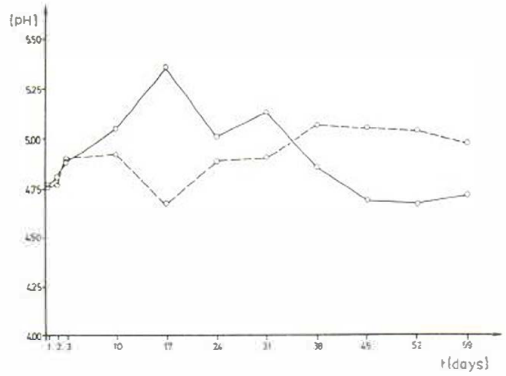

Fig. 1. Development of the mean pH values at the forehead in the two study groups (——, "Lux" group; --- "Sebamed" group).

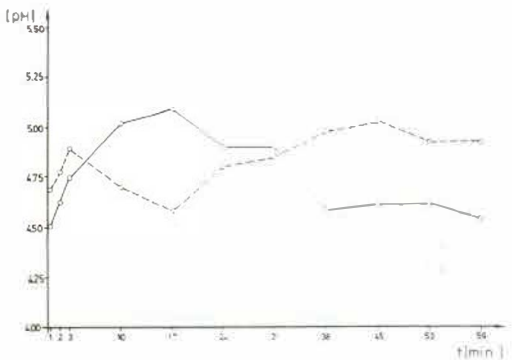

Fig. 2. Development of the mean pH values at the forearm in the two study groups (——, "Lux" group; ---, "Sebamed" group).

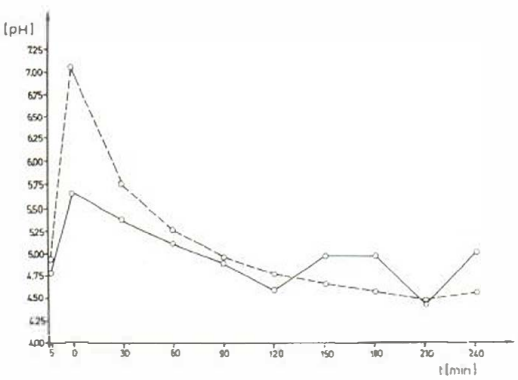

Fig. 3. Short-term development of the mean pH values at the forehead after application of the cleansing agent (---, soap; ——, synthetic detergents) in the steady-state.

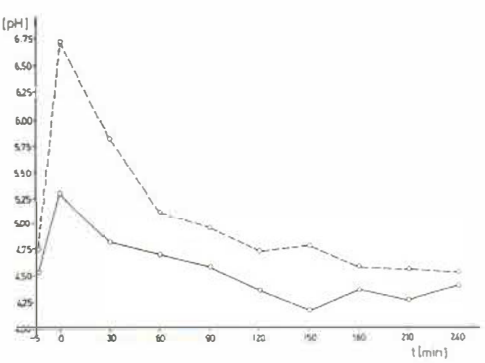

Fig. 4. Short-term development of the mean pH values at the forearm after application of the cleansing agent (---, soap; ——, synthetic detergents) in the steady-state.

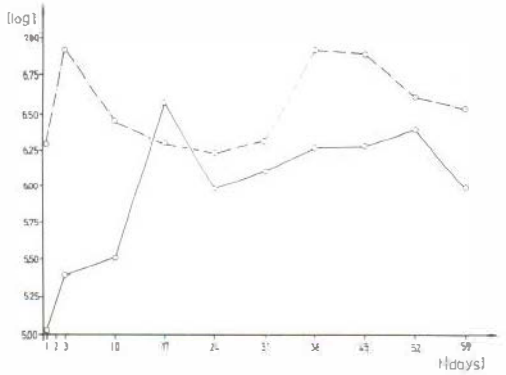

Fig. 5. Development of the counts of coagulase-negative staphylococci per square centimetre at the forehead in the two study groups (——, "Lux" group; ---, "Sebamed" group).

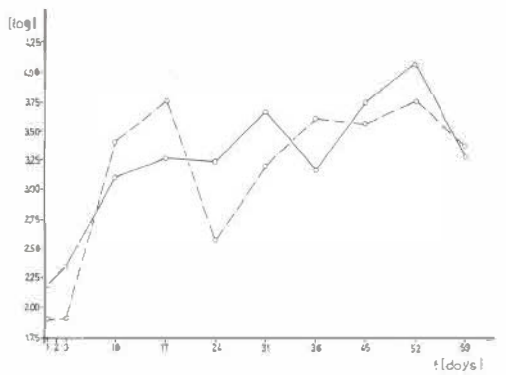

Fig. 6. Development of the counts of coagulase-negative staphyloccoci per square centimetre at the forearm in the two study groups (——, "Lux" group; --- "Sebamed" group).

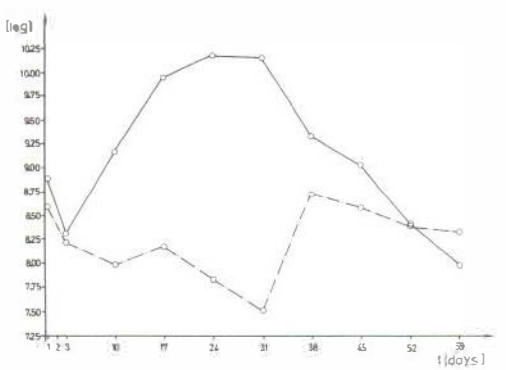

Fig. 7. Development of the counts of propionibacteria per square centimetre at the forehead in the two study groups (——, "Lux" group; ---, "Sebamed" group).

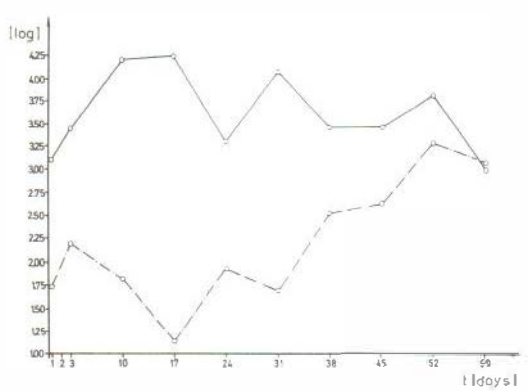

Fig. 8. Development of the counts of propionibacteria per square centimetre at the forearm in the two study groups (——, "Lux" group; --- "Sebamed" group).

section of Material and Methods) (16, 17). With respect to the pioneering paper of Schade & Marchionini (16) the difference between the given and the true value amounted to up to 0.7 units. The repeated determination of individual pH values at the skin sites investigated on consecutive days showed no major differences. Moreover there were no or almost no differences between the mean pH values in the two study groups.

The short-time effects of washing the skin with soap or synthetic detergents on its pH reported in the literature (1, 4) are in agreement with the present findings: 1) Washing the skin once with soap makes the skin more alkaline than washing it with synthetic detergents. 2) The effect of the single application of a cleansing agent only lasts for several hours. The long-term effects of often repeated washings with soap and synthetic detergents on the skin pH, however, have not systematically been investigated so far. Due to the present data under such conditions the mean skin pH does seem to be open to long-lasting alterations which may have consequences on other features of the skin. Previous investigations of the effect of washing the skin with cleasing agents on its bacterial flora have mainly been focussed on the effect of a single washing procedure on the aerobic flora (5, 18). Although a certain influence of the washing procedure in general could be proven as well as differential effects of different cleansing agents, no general clear-cut tendency of striking character can be derived from these data. Very much the same holds true of our findings with respect to the behaviour of coagulase-negative staphylococci under repeated washings with soap or synthetic detergents. They are in good accordance with the results of Hartmann (19), whose study is comparable to a certain extent. With respect to the propionibacteria, however, the situation looks totally different: the counts of the bacteria are as impressively increased in the presence of soap as they are depressed in the presence of synthetic detergents. This may explain why repeated synthetic detergents washings seem to be helpful in the treatment of acne vulgaris in whose pathogenesis these bacteria play a major role (20).

The possible interdependence of the bacterial flora and the pH of the skin was already seen by Marchionini and co-workers (7). Later on this theory has especially been supported by Arnold (2), who experimentally demonstrated the effect of acidification and alkalinization of the skin on its bacterial flora. In connection with (repeated) washing procedures the development of skin pH and flora had so far not been investigated together over a longer period. Analysis of the pertinent data acquired in the present investigation in parallel shows in fact a clear correlation between these two features of the skin. It is tempting to speculate that the increase of the propionibacteria counts in soap washings and its decrease in synthetic detergents washings is caused by the shift in the skin surface pH induced by such procedures. Sure enough such a hypothesis will need further confirmation by other studies with a different experimental design.

REFERENCES

1. Peukert L. Einfluß der Titrationsalkalität von Reinigungsmitteln auf den pH-Wert der menschlichen Haut. Arch Dermatol Syph 1941; 181: 417–424.
2. Arnold L. Relationship between certain physico-chemical changes in the cornified layer and the endogenous bacterial flora of the skin. J Invest Dermatol 1942; 5: 207–223.
3. Burckhardt W. Methoden zur Untersuchung der Wirkung synthetischer Waschmittel auf die Haut. Dermatologica 1964; 129: 37–46.
4. Pöls H, Schirren CG. Beeinflussung des pH-Wertes der Hautoberfläche durch Seifen, Waschmittel und synthetische Detergentien. Hautarzt 1966; 17: 37–40.
5. Hartmann AA, Röckl H. Vergleichende Untersuchung über den Einfluß von Balneum Hermal Gel zum Duschen auf die aerobe Residentflora der Haut bei einmaliger Anwendung. Ärztl Kosmetol 1979; 9: 16–25.
6. Holt RJ. Aerobic bacterial counts on human skin after bathing. J Med Microbiol 1971; 4: 319–327.

7. Marchionini A, Hausknecht W. Säuremantel der Haut und Bakterienabwehr. Klin Wochenschr 1938; 17: 663–666.
8. Röckl H. Probleme der Bakterienökologie der Haut. Hautarzt 1977; 28: 155–159.
9. Schirren CG. Does the glass-electrode determine the same pH values on the skin surface as the quinhydrone electrode? J Invest Dermatol 1955; 24: 485-488.
10. Arbenz H. Untersuchungen über die pH-Werte der normalen Hautoberfläche. Dermatologica 1952; 105: 133–153.
11. Williamson P, Kligman AM. A new method for the quantitative investigation of cutaneous bacteria. J Invest Dermatol 1965; 45: 498–503.
12. Hartmann AA. A comparative investigation of methods for sampling skin flora. Arch Dermatol Res 1982; 274: 381–385.
13. Kloos WE, Schleifer KH. Simplified scheme for routine identification of human staphylococcus species. J Clin Microbiol 1975; 1: 82–88.
14. Nord CE, Dahlbäck A, Wadström T. Evaluation of a test kit for identification of anaerobic bacteria. Med Microbiol Immunol 1973; 161: 239–242.
15. Essers L, Haralambie E. Experiences with the API 20A System in routine species identification of anaerobes. Zbl Bact Hyg I Abt Orig A 1977; 238: 394–401.
16. Schade H, Marchionini A. Der Säuremantel der Haut (nach Gaskettenmessungen). Klin Wochenschr 1928; 7: 12–14.
17. Jolly HW, Hailey CW, Netick J. pH determinations of the skin. J Invest Dermatol 1961; 36: 305–308.
18. Hartmann AA. Duschbaden und sein Einfluß auf die aerobe Residentflora der menschlichen Haut. Arch Dermatol Res 1980; 267: 161–174.
19. Hartmann AA. Tägliches Baden und Verhalten der Hautflora. Quantitative und qualitative Untersuchungen der aeroben Hautflora. Arch Dermatol Res 1979; 265: 153–164.
20. Reichert U, Saint-Leger D, Schaefer H. Skin surface chemistry and microbial infection. Semin Dermatol 1982; 1: 91–99.